



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 5/10 // C07K 14/47	A1	(11) International Publication Number: WO 99/58646 (43) International Publication Date: 18 November 1999 (18.11.99)
(21) International Application Number: PCT/EP99/03075 (22) International Filing Date: 5 May 1999 (05.05.99) (30) Priority Data: MI98A001004 8 May 1998 (08.05.98) IT (71) Applicant (for all designated States except US): GENERA S.P.A. [IT/IT]; Via Olgettina, 58, I-20132 Milano (IT). (72) Inventor; and (75) Inventor/Applicant (for US only): MAVILIO, Fulvio [IT/IT]; Via Olgettina, 58, I-20132 Milano (IT). (74) Agent: MINOJA, Fabrizio; Bianchetti Bracco Minoja S.r.l., Via Rossini, 8, I-20122 Milano (IT).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: GENETICALLY-MODIFIED FIBROBLASTS AND THE USE THEREOF		
(57) Abstract <p>A method for the preparation of genetically-modified fibroblasts expressing a muscle lineage commitment gene, and the use thereof for the treatment of genetic defects or for the expression of therapeutic proteins.</p>		



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CLAIMS

1. A method for the preparation of genetically-modified fibroblasts expressing a muscle lineage commitment gene, which comprises:
- 5 - a) ex-vivo transduction of fibroblasts with a therapeutic gene or a gene capable of correcting a gene defect;
- b) transient expression of the muscle lineage commitment gene in fibroblasts transduced as at point (a), through transformation of the cells with a high-efficiency DNA transfer method, wherein the
- 10 muscle lineage commitment gene is under the control of a strong promoter.
2. A method according to claim 1, wherein the therapeutical gene is the dystrophin gene.
3. A method according to claim 1, wherein the high-efficiency DNA
- 15 transfer method is a viral vector.
4. A method according to claim 3, wherein said viral vector is selected from baculovirus, adeno-related viruses, adeno-virus.
5. A method according to claim 3, wherein said vector is an adenovirus.
- 20 6. A method according to claim 1, wherein the muscle lineage commitment gene is selected from MyoD, Myf-5, MRF4 and myogenin.
7. A method according to claim 6, wherein said gene is MyoD.
8. A method according to claim 1, wherein the muscle lineage commitment gene is under the control of a viral promoter.
- 25 9. Genetically-modified fibroblasts transiently expressing a muscle lineage commitment gene.
10. Fibroblasts according to claim 9, wherein the muscle lineage

commitment gene is MyoD.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/03075

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N5/10 //C07K14/47

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 12979 A (UNIV SOUTHERN CALIFORNIA) 18 May 1995 (1995-05-18) cited in the application the whole document	9, 10
Y	page 14, line 10 - page 15, line 4 ---	1-10
Y	WO 96 09373 A (BRITISH TECH GROUP ;WATT DIANA JOAN (GB); BETTENCOURT DE MEDEIROS) 28 March 1996 (1996-03-28) cited in the application the whole document ---	1-10
A	WO 98 17784 A (UNIV LAVAL ;TREMBLAY JACQUES P (CA)) 30 April 1998 (1998-04-30) the whole document --- -/--	



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

23 August 1999

Date of mailing of the international search report

13/09/1999

Name and mailing address of the ISA

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/03075

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 219 740 A (MILLER A DUSTY ET AL) 15 June 1993 (1993-06-15) the whole document ---	
A	WEINTRAUB H ET AL: "ACTIVATION OF MUSCLE-SPECIFIC GENES IN PIGMENT, NERVE, FAT, LIVER, AND FIBROBLAST CELL LINES BY FORCED EXPRESSION OF MYOD" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 86, 1 July 1989 (1989-07-01), pages 5434-5438, XP000672860 ISSN: 0027-8424 the whole document ---	
P,X	LATTANZI L, ET AL.: "High Efficiency Myogenic Conversion of Human Fibroblasts by Adenoviral Vector-Mediated MyoD gene transfer" JOURNAL OF CLINICAL INVESTIGATION, vol. 101, no. 10, 15 May 1998 (1998-05-15), pages 2119-2128, XP002112711 page 2124 - page 2125 page 2127, column 2, paragraph 1 ---	1-10
P,X	HUARD C, ET AL.: "Transplantation of Dermal Fibroblasts Expressing MyoD1 in Mouse Muscles " BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 248, no. 3, 30 July 1998 (1998-07-30), pages 648-654, XP002112712 page 651, column 2, paragraph 1 -----	1-10

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte: onal Application No

PCT/EP 99/03075

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9512979 A	18-05-1995	AU 1052795 A	29-05-1995
WO 9609373 A	28-03-1996	AU 694957 B	06-08-1998
		AU 3481695 A	09-04-1996
		CA 2198379 A	28-03-1996
		EP 0783568 A	16-07-1997
		GB 2293604 A,B	03-04-1996
		JP 10505756 T	09-06-1998
WO 9817784 A	30-04-1998	AU 4613397 A	15-05-1998
US 5219740 A	15-06-1993	NONE	

PATENT COOPERATION TREATY

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REC'D 18 AUG 2000

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

16

Applicant's or agent's file reference SCB 479 PCT	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/EP99/03075	International filing date (day/month/year) 05/05/1999	Priority date (day/month/year) 08/05/1998
International Patent Classification (IPC) or national classification and IPC C12N5/10		
Applicant GenEra S.p.A. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.



2. This REPORT consists of a total of 5 sheets, including this cover sheet.

- ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 1 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 28/10/1999	Date of completion of this report 14. 08. 00
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Bilang, J Telephone No. +49 89 2399 8707 

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/EP99/03075

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-22 as originally filed

Claims, No.:

1-10 with telefax of 10/05/2000

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

see separate sheet

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-10
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-10
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-10
	No:	Claims	

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/EP99/03075

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP99/03075

Additional remarks Item I

The priority for the present application is validly claimed. The P documents cited in the International Search Report therefore need not to be considered for the assessment of novelty and inventive step.

Additional remarks Item V

1. The present application discloses a method for the preparation of genetically modified fibroblasts. The method comprises the steps of (a) transducing the fibroblasts with a therapeutic gene or a gene capable of correcting a gene defect, and b) transiently expressing a muscle lineage commitment gene through transformation of the fibroblasts of step (a).
2. The following documents were taken into consideration:
 - 2.1 D1 (WO 95/12979; cited in the application) discloses a method for the conversion of proliferating fibroblasts into myoblasts. For this purpose the fibroblasts are transformed with MyoD1, a myogenic determination gene.
 - 2.2 D2 (WO 96/09373; cited in the application as GB 2 293 604) discloses a method for the preparation of fibroblasts suitable for the treatment of muscular disorders such as dystrophy. The fibroblasts may be transformed with a therapeutic gene such as dystrophin (Example 8) and are implanted in muscles of the recipient. After implantation the fibroblasts convert to myogenic cells.
3. None of the available prior art documents discloses or suggests a method for the preparation of genetically modified fibroblasts comprising the step of transiently expressing a muscle lineage commitment gene in the fibroblast. In contrast to the method disclosed in D1, it also appears that the fibroblasts used in the method of the present invention are not proliferating.

Moreover, the present method apparently leads to a much higher conversion to myoblasts than the prior art.

The subject-matter of claims 1-10 thus can be considered to be novel (Article 33(2) PCT) and based on an inventive activity in the sense of Article 33(3) PCT.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP99/03075

Additional remarks Item VIII

It appears to be a characterizing feature of the present application that the method is performed on non-proliferating fibroblasts. The claims, however, do not refer to this feature.

It also appears from the description that the expression of the muscle lineage commitment gene occurs ex-vivo (examples). Claim 1, however, does not clearly refer to this feature of the claimed method.

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

**Assistant Commissioner for Patents
United States Patent and Trademark
Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE**

in its capacity as elected Office

Date of mailing (day/month/year) 02 December 1999 (02.12.99)	in its capacity as elected Office
International application No. PCT/EP99/03075	Applicant's or agent's file reference scb 479 PCT
International filing date (day/month/year) 05 May 1999 (05.05.99)	Priority date (day/month/year) 08 May 1998 (08.05.98)
Applicant MAVILIO, Fulvio	

- 1. The designated Office is hereby notified of its election made:**

☒ in the demand filed with the International Preliminary Examining Authority on:

28 October 1999 (28.10.99)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

<p>The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland</p> <p>Facsimile No.: (41-22) 740.14.35</p>	<p>Authorized officer</p> <p>F. Baechler</p> <p>Telephone No.: (41-22) 338.83.38</p>
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/12912

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A01N 43/04; A61K 31/70; C07H 17/00; C12N 15/00

US CL : 435/ 172.1, 172.3, 320.1; 514/44; 536/ 23.1, 23.5

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/ 172.1, 172.3, 320.1; 514/44; 536/ 23.1, 23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CA, BIOSIS, EMBASE, MEDLINE, DERWENT BIOTECHNOLOGY ABSTRACTS

search terms: MyoD, retrovirus, infarction, heart

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	Cell, Volume 51, issued December 1987, R.L. Davis et al., "Expression of a Single Transfected cDNA Converts Fibroblasts to Myoblasts", pages 987-1000, see the entire document.	1-3, 6-8 ----- 4, 5
Y	Proceedings of the National Academy of Science, USA, Volume 88, issued May 1991, R.N. Kitsis et al., "Hormonal modulation of a gene injected into rat heart <i>in vivo</i> ", pages 4138-4142, see the entire document.	1-8
Y	Development, Volume 114, issued 1992, J.H. Miner et al., "Skeletal muscle phenotypes initiated by ectopic MyoD in transgenic mouse heart", pages 853-860, see the entire document.	1-8



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	&	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

08 DECEMBER 1994

Date of mailing of the international search report

JAN 24 1995

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
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Authorized officer

BRUCE CAMPELL



C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	Circulation, Volume 88, No. 4 part 2, issued 08 November 1993, H. Prentice et al., "Transformation of Cardiac Fibroblasts into the Skeletal Muscle Phenotype by Injection of a MyoD-Expressing Retrovirus into Ischemic Heart", page I-475, see the abstract.	1-8
Y	Circulation Research, Volume 72, issued 1993, R. von Harsdorf et al., "Gene Injection Into Canine Myocardium as a Useful Model for Studying Gene Expression in the Heart of Large Mammals", pages 688-695, see the entire document.	1-8
A	Science, Volume 261, issued 15 February 1991, H. Weintraub et al., "The myoD Gene Family: Nodal Point During Specification of the Muscle Cell Lineage", pages 761-766, see the entire document.	1-8
Y	Circulation, Volume 82, No. 6, issued December 1990, H. Lin et al., "Expression of Recombinant Genes in Myocardium <i>In Vivo</i> After Direct Injection of DNA", pages 2217-2221, see the entire document.	1-8



PATENT COOPERATION TREATY



PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference SCB 479 PCT	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/EP 99/ 03075	International filing date (day/month/year) 05/05/1999	(Earliest) Priority Date (day/month/year) 08/05/1998
Applicant GenEra S.p.A.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☒ None of the figures.

- 35 -

5. Austin L and Burgess AW: Stimulation of myoblast proliferation in culture by leukaemia inhibitory factor and other cytokines. J Neurol. Sci 1991;101: 193-197.
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5 C, Ishihara T, Nonaka I, Ozawa E and Sugita H:
Immunostaining of skeletal and cardiac muscle membrane
with antibody against Duchenne muscular dystrophy
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10 Quantitative studies of the efficacy of myoblast
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Law PK: Dystrophin cytochemistry in mdx mouse muscle
injected with labeled normal myoblasts. Cell Transpl
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- 10 13. DiMario J, Buffinger N, Yamada S and Strohman RC: Fibroblast growth factor in the extracellular matrix of dystrophic (mdx) mouse muscle. Science 1989; 244: 688-690.
- 15 14. DiMario J and Strohman RC: Satellite cells from dystrophic (mdx) mouse muscle are stimulated by fibroblast growth factor in vitro. Differentiation 1988; 39: 42-49.
- 20 15. Florini JR and Magri KA: Effect of growth factors on myogenic differentiation. Am J Physiol 1989; 256: C701-C711.

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17. Gussoni E, Pavlath PK, Lanctot AM, Sharma K, Miller RG, Steinman L and Bland HM: Normal dystrophin
5 transcripts detected in DMD patients after myoblast transplantation. Nature 1992; 356: 435-438.
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10 Development 1993;119: 691-701.
19. Hoffman EP, Brown RH and Kunkel LM: Dystrophin: the protein product of Duchenne muscular dystrophy locus. Cell 1987; 51: 919-928.
20. Hsu H-Y, Nicholson AC and Hajjar DP: Basic
15 Fibroblast Growth Factor-induced Low Density Lipoprotein Receptor Transcription and Surface Expression. J. Biol. Chem. 1994; 269: 9213-9220.
21. Huard J, Ascadi G, Jani A, Massi B: Gene transfer into mdx skeletal muscle by isogenic, genetically

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labelled myoblasts. Muscle and Nerve, Suppl. 1, S260, 1994.

22. Huard J, Bouchard JP, Roy R, Malouin F, Dansereau G, Labrecque C, Albert N, Richards CL, Lemieux B and
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WHAT IS CLAIMED IS:

1. A method for increasing the number of transplanted functional donor's myoblasts which are fused with non-functional myoblasts of a recipient individual suffering of a myopathy, which comprises the
5 step of growing *in vitro* said donor's myoblasts in a appropriate culture medium in the presence of fibroblasts and of an agent inducing the secretion of an enzyme involved in extracellular matrix destruction,
10 prior to injecting a mixture comprising said donor's myoblasts and induced enzyme into said recipient individual's muscle, whereby a functional muscle is at least in part restored.

2. A method for increasing the number of transplanted functional donor's myoblasts which are
15 fused with non-functional myoblasts of a recipient individual suffering of a myopathy, which comprises the steps of: inserting into said donor's myoblasts a gene construct capable of expressing an enzyme involved in
20 extracellular matrix destruction, obtaining thereby recombinant donor's myoblasts, and growing said recombinant donor's myoblasts in an appropriate culture medium, prior to injecting said recombinant donor's

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myoblasts into said recipient individual's muscle, whereby a functional muscle is at least in part restored.

3. A method of claim 1, wherein said
5 enzyme is a metalloprotease.

4. A method of claim 2, wherein said enzyme is a metalloprotease.

5. A method of claim 3, wherein said metalloprotease is Gelatinase A or Matrilysine.

10 6. A method of claim 4, wherein said metalloprotease is Gelatinase A or Matrilysine.

7. A method of claim 1, 3 or 5, wherein said agent is Concanavalin A or phorbol ester.

15 8. A method as defined in any one of claims 1 to 7, wherein said culture medium further comprises a growth or trophic factor for increasing the multiplication of said donor's myoblasts.

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9. A method as defined in claim 8,
wherein said growth or trophic factor is selected from
the group consisting of basic fibroblast growth factor
(bFGF), insulin growth factor I, transferrin,
5 platelet-derived growth factor, epidermal growth factor,
adrenocorticotrophin, macrophage colony-stimulating
factor, protein kinase C activators, agonists thereof,
and combinations thereof.

10. A method as defined in claim 9,
10 wherein said factor is bFGF.

11. A method as defined in any one of
claims 1, 3, 5, 7, 8, 9, and 10, wherein said donor's
myoblasts are obtained for a primary myoblast culture
resulting from culturing a cell dispersion of donor's
15 muscle biopsy.

12. A method as defined in claim 11,
wherein said primary myoblast culture is grown in the
presence of 100 ng of recombinant human basic fibroblast
growth factor per milliliter of culture medium for a
20 period of time of about 48 hours before transplantation.

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13. A method as defined in claim 11,
wherein said primary myoblast culture is grown in the
presence of 100 ng of recombinant human basic fibroblast
growth factor and 20 μ g Concanavalin A per milliliter of
5 culture medium for a period of time of about 48 hours
before transplantation.

14. A method as defined in any one of
claims 1 to 13, wherein said myopathy is Duchenne
muscular dystrophy.

10 15. A method for increasing the number of
transplanted functional donor's cells which are fused
with corresponding non-functional cells of a recipient
individual's tissue, which comprises the steps of:
inserting into said donor's cells a gene construct
15 capable of expressing an enzyme involved in
extracellular matrix destruction, obtaining thereby
recombinant donor's cells, and growing said recombinant
donor's cells in an appropriate culture medium, prior to
injecting said recombinant donor's cells into said
20 recipient individual's tissue, whereby a functional
tissue is at least in part restored.

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16. A method of claim 15, wherein said enzyme is a metalloprotease.

17. A method of claim 16, wherein said metalloprotease is Matrilysine or Gelatinase A.

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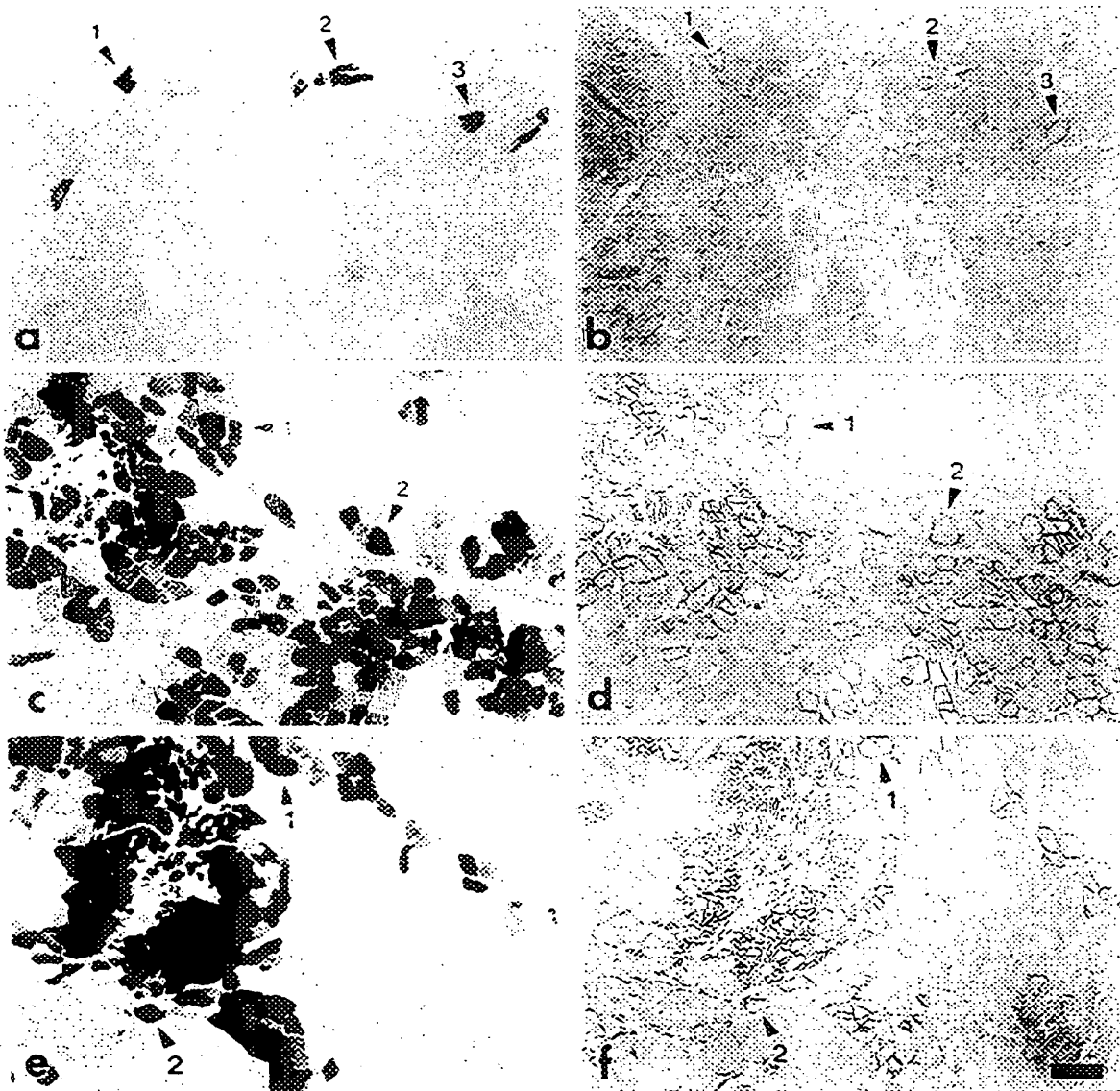


FIG. 1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 97/00774

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9628541 A	19-09-96	AU 4934496 A EP 0815205 A	02-10-96 07-01-98
WO 9112329 A	22-08-91	AU 7312891 A US 5466676 A	03-09-91 14-11-95
US 5435999 A	25-07-95	AT 138267 T AU 624284 B AU 6896791 A WO 9107992 A CA 2045630 A DE 69027123 D DE 69027123 T EP 0502081 A	15-06-96 04-06-92 26-06-91 13-06-91 25-05-91 27-06-96 24-04-97 09-09-92

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claims 1-17 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 97/00774

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Inte. onal Application No
PCT/CA 97/00774

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>CRINNION J. N. ET AL.: "Role of neutrophil-endothelial adhesion in skeletal muscle reperfusion injury" BRITISH JOURNAL OF SURGERY, vol. 83, no. 2, February 1996, pages 251-254, XP002054086 cited in the application see the whole document</p> <p>---</p>	1-17
A	<p>POWELL W.C.: "Expression of the Metalloproteinase Matrilysin in DU-145 Cells Increases Their Invasive Potential in severe Combined Immunodeficient Mice" CANCER RESEARCH, vol. 53, no. 2, 15 January 1993, pages 417-422, XP002054097 cited in the application see the whole document</p> <p>-----</p>	2-17

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 97/00774

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N5/08 C12N5/10 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96 28541 A (UNIVERSITE LAVAL) 19 September 1996 see the whole document	1-17
A	KINOSHITA I ET AL: "Pretreatment of myoblast cultures with basic fibroblast growth factor increases the efficacy of their transplantation in mdx mice." MUSCLE & NERVE 18 (8). 1995. 834-841, XP000572291 see the whole document	1-17
A	WO 91 12329 A (BOARD OF THE REGENTS, THE UNIVERSITY OF TEXAS) 22 August 1991 see the whole document	1-17
A	US 5 435 999 A (AUSTIN L.) 25 July 1995 see the whole document	1-17
-/-		

☒ Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search

2 February 1998

Date of mailing of the international search report

23.02.98

Name and mailing address of the ISA

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Moreau, J

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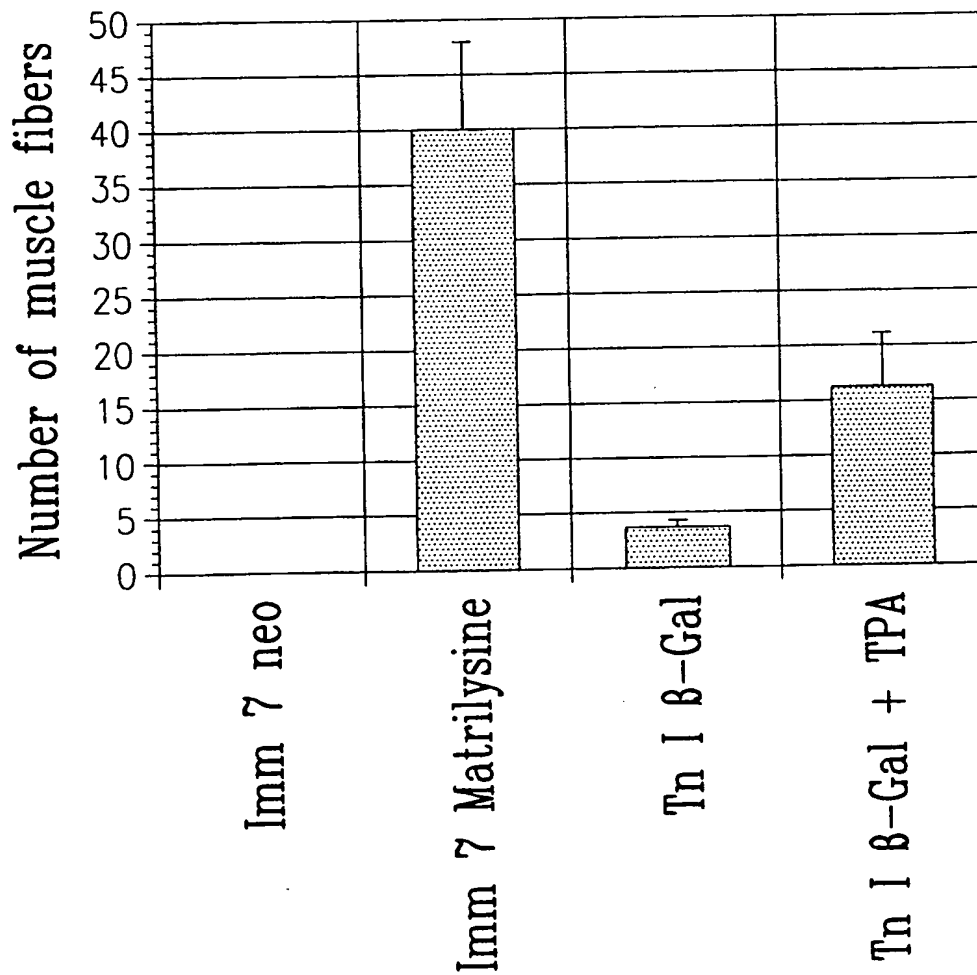


Figure 2

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/03075

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N5/10 //C07K14/47

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 12979 A (UNIV SOUTHERN CALIFORNIA) 18 May 1995 (1995-05-18) cited in the application the whole document	9,10
Y	page 14, line 10 - page 15, line 4 ---	1-10
Y	WO 96 09373 A (BRITISH TECH GROUP ;WATT DIANA JOAN (GB); BETTENCOURT DE MEDEIROS) 28 March 1996 (1996-03-28) cited in the application the whole document ---	1-10
A	WO 98 17784 A (UNIV LAVAL ;TREMBLAY JACQUES P (CA)) 30 April 1998 (1998-04-30) the whole document --- -/--	

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

23 August 1999

Date of mailing of the international search report

13/09/1999

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/03075

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 219 740 A (MILLER A DUSTY ET AL) 15 June 1993 (1993-06-15) the whole document ---	
A	WEINTRAUB H ET AL: "ACTIVATION OF MUSCLE-SPECIFIC GENES IN PIGMENT, NERVE, FAT, LIVER, AND FIBROBLAST CELL LINES BY FORCED EXPRESSION OF MYOD" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 86, 1 July 1989 (1989-07-01), pages 5434-5438, XP000672860 ISSN: 0027-8424 the whole document ---	
P, X	LATTANZI L, ET AL.: "High Efficiency Myogenic Conversion of Human Fibroblasts by Adenoviral Vector-Mediated MyoD gene transfer" JOURNAL OF CLINICAL INVESTIGATION, vol. 101, no. 10, 15 May 1998 (1998-05-15), pages 2119-2128, XP002112711 page 2124 - page 2125 page 2127, column 2, paragraph 1 ---	1-10
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INTERNATIONAL SEARCH REPORT

In relation on patent family members

International Application No

PCT/EP 99/03075

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
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			AU	3481695 A	09-04-1996
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